

Original Article

The association between VEGF -634C/G polymorphisms and osteonecrosis of femoral head: a meta-analysis

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Abstract: The polymorphism of the vascular endothelial growth factor (VEGF) -634C/G has been correlated with susceptibility to osteonecrosis of the femoral head (ONFH). The aim of this study was to derive a more precise estimation of the relationship between the VEGF -634C/G polymorphism and ONFH by performing a meta-analysis. We searched articles indexed in Pubmed, OVID and Web of Science published up to January 2015 that met our predefined criteria. The strength of the association between VEGF -634C/G polymorphism and ONFH risk was assessed by an odds ratio (OR) with the corresponding 95% CI. Three eligible studies involving 692 cases and 875 controls were identified. Overall, pooled analysis indicated a significant association between VEGF -634C/G polymorphism and ONFH risk (for C vs. G: OR=1.141, 95% CI 1.055-1.235, $P=0.001$; for CC vs. GG: OR=1.345, 95% CI 1.124-1.610, $P=0.001$; for CG vs. GG: OR=1.106, 95% CI 1.018-1.202, $P=0.017$; for CG+CC vs. GG: OR=1.104, 95% CI 1.035-1.177, $P=0.003$; for CC vs. GG+ CG: OR=1.294, 95% CI 1.051-1.593, $P=0.015$). No evidence of publication bias was observed. In conclusion, this meta-analysis suggested that polymorphism of VEGF -634C/G was a risk factor for ONFH. This finding needs further confirmation by trans-regional multicenter study with large sample in different ethnic populations, such as Caucasian and Austroloid.

Keywords: Vascular endothelial growth factor, polymorphism, osteonecrosis, risk factor, meta-analysis

Introduction

Osteonecrosis of the femoral head (ONFH) is an ischemic injury that results in necrosis of the subchondral bone, collapse of the femoral head, and degeneration of the hip [1]. Although ONFH can be caused by several conditions, such as trauma, glucocorticoid therapy, alcoholism, and storage diseases, the pathogenesis of ONFH is not yet completely understood [2-4].

It is reported that angiogenesis is very important for the bone repair process, and the association between blood vessels and bone repair has already been recognized [5]. In an animal study, the vascular endothelial growth factor (VEGF) gene transfer resulted in the enhanced neovascularization of the necrotic bone in a rabbit model of ON [6, 7]. Radke et al. find that VEGF is significantly highly expressed in the edematous area of the ON adjacent to the necrotic area [8], indicating that there is a significant association between VEGF expression

with bone tissue repair [9]. VEGF is pivotal for bone formation, which includes blood vessel invasion and cartilage remodeling [10]. VEGF has also been implicated in bone repair [11]. The VEGF gene was reported to be polymorphic, especially in the promoter region (-2578, -1154, etc.), the 5'-untranslated region (UTR) (-634, -7) and in the 3'-UTR (+936). Several studies have shown that polymorphisms within the 5'-UTR have lead to differences in VEGF expression, and that they could influence the etiology of a variety of pathological conditions such as diabetic retinopathy [12, 13], prostate cancer [14], and breast cancer [15]. Recent studies have reported an association between ONFH and genetic polymorphisms in VEGF -634C/G leading to angiogenesis disorders [16-18]. Kim et al. reported a statistically significant association between the incidence of ONFH and the VEGF -634C/G gene mutation in 312 patients [16]. Lee et al. demonstrated a similar result in 160 patients [17]. Liu et al. suggested that the VEGF -634C/G polymorphism plays a role in the

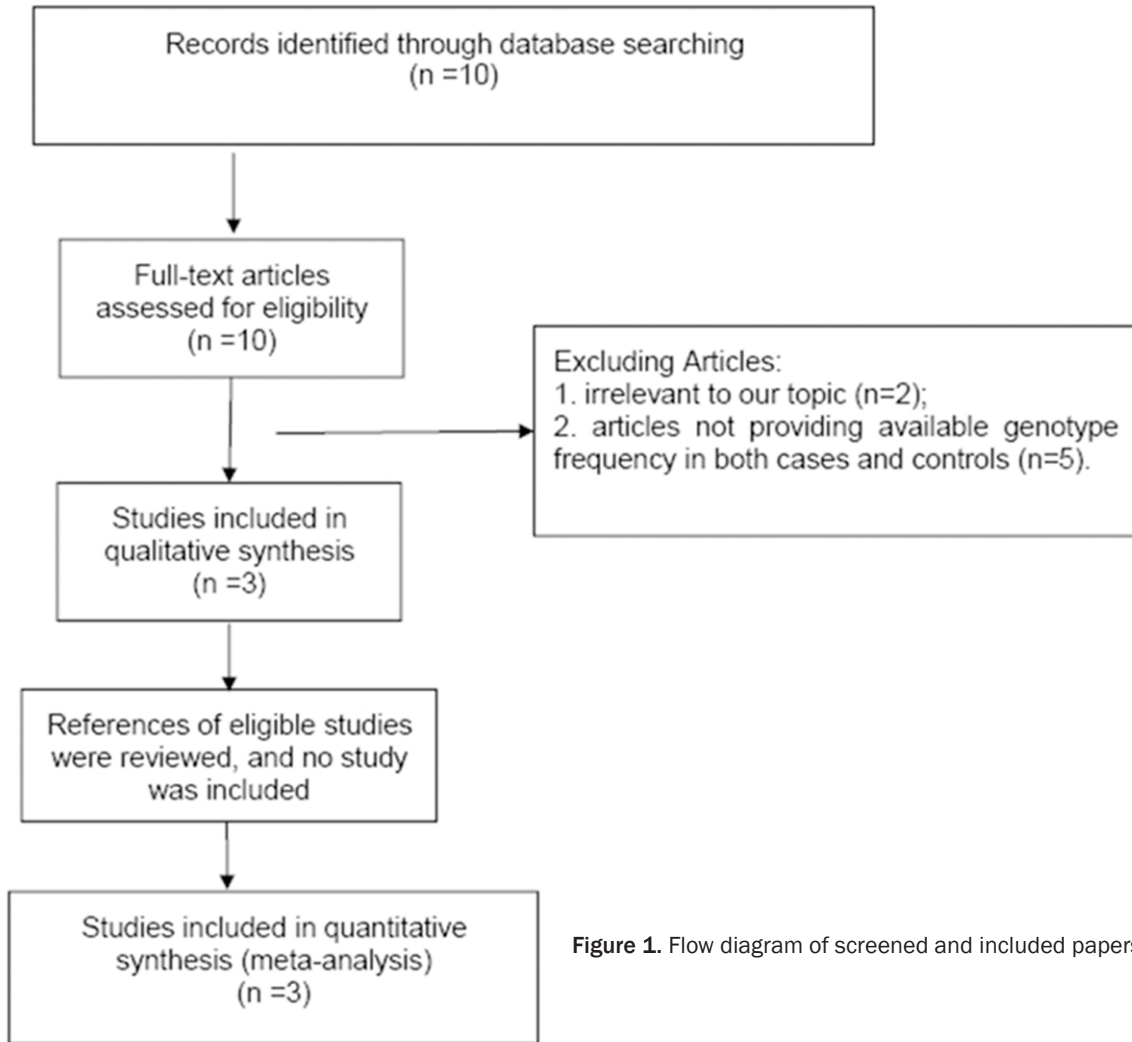


Figure 1. Flow diagram of screened and included papers.

Table 1. Characteristics of subjects in eligible studies

Studies	Country	Detection-method	ONFH					Healthy control					HWE	Quality score
			No.	CC	CG	GG	C-(frequency)	No.	CC	CG	GG	C-(frequency)		
Kim 2008	Korea	Taqman assay	312	58	171	83	0.460	495	80	243	172	0.407	Yes	7
Lee 2012	Korea	PCR-RFLP	160	37	89	34	0.509	160	27	86	47	0.436	Yes	7
Liu 2012	China	PCR-RFLP	220	56	103	61	0.489	220	38	112	70	0.427	Yes	7

PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism.

pathogenesis of ONFH in 220 Chinese patients [18]. Meta-analysis is a well established statistical tool that serves for integration of data from independent studies in order to formulate more general conclusions. The aim of this study was to assess the association of the VEGF -634C/G gene polymorphism with the risk of ONFH by conducting a meta-analysis of individual datasets from all eligible studies published to date.

Methods

Search strategy

We searched all articles indexed in Pubmed, OVID and Web of Science published up to January 2015. Literature searches were performed using medical subject heading (MeSH) or free text words. The searching keywords were: ("vascular endothelial growth factor" OR VEGF) AND polymorphism AND osteonecrosis.

VEGF-634C/G polymorphisms and osteonecrosis

Table 2. Meta-analysis of the association between VEGF -634C/G polymorphism and ONFH

Genetic models	Test of association			Heterogeneity		Effects model
	OR	95% CI	P	I ²	P	
C vs. G	1.141	1.055-1.235	0.001	0.0%	0.957	Fix-effect model
CC vs. GG	1.345	1.124-1.610	0.001	0.0%	0.915	Fix-effect model
CG vs. GG	1.106	1.018-1.202	0.017	0.0%	0.509	Fix-effect model
CG+CC vs. GG	1.104	1.035-1.177	0.003	0.0%	0.738	Fix-effect model
CC vs. GG+CG	1.294	1.051-1.593	0.015	0.0%	0.574	Fix-effect model

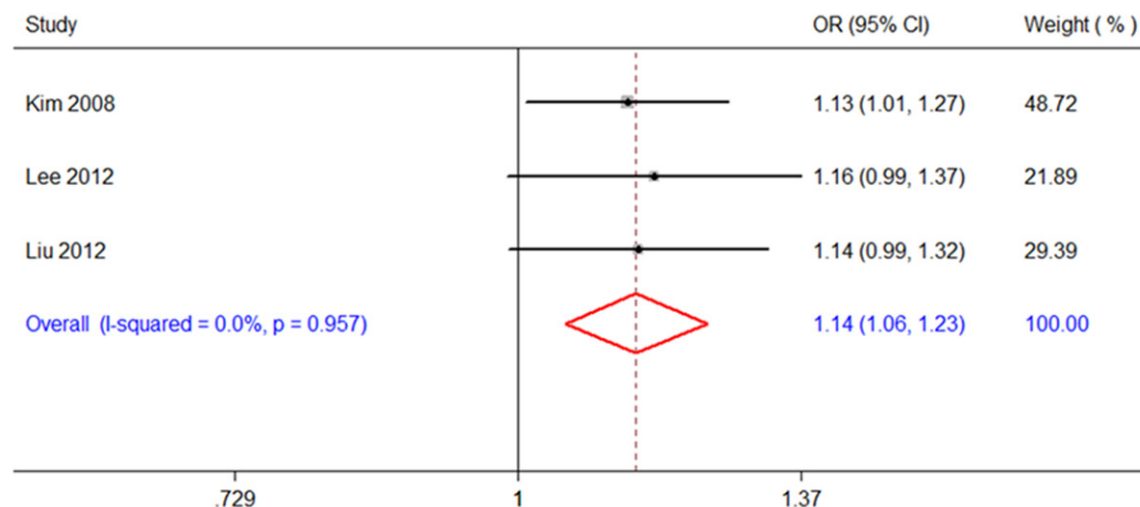


Figure 2. Forest plot of ORs in the additive model (C vs. G) for the association between VEGF -634C/G polymorphism and ONFH risk. The combined OR and 95% confidence intervals (CIs) were calculated using the fix-effects model.

Reference lists of all eligible studies were screened to identify potentially eligible studies. Emails were sent to the authors of identified studies for additional information if necessary. We accepted studies written in English.

Selection criteria

Three authors conducted the search independently. Titles and abstracts were screened for subject relevance. Studies that could not be definitely excluded based on abstract information were also selected for full text screening. Two authors independently selected eligible studies for inclusion possibility. Where there was a disagreement for study inclusion, a discussion was held to reach a consensus. Eligible studies had to meet the following criteria: (1) human study; (2) case-control study; (3) articles evaluating the association between VEGF -634C/G polymorphism and ONFH risk; (4) studies providing available genotype frequency in both cases and controls; (5) sufficient data

available to estimate an odds ratio (OR) with its 95% CI; (6) there was no deviation from Hardy-Weinberg equilibrium (HWE) among the controls.

Data extraction and quality assessment

Information was carefully extracted from all eligible publications independently by two authors according to the inclusion criteria listed above. The following information was extracted from each included study: first author's family name, year of publication, country, total numbers of cases and controls, genotyping method and genotyping information.

The qualities of all included studies were assessed using the Newcastle-Ottawa Scale (NOS) [19]. The assessment tool focused on three aspects, including participant selection, comparability and exposure. The studies would be assigned stars of 9 if all items were satisfied. Two authors assessed the quality independently.

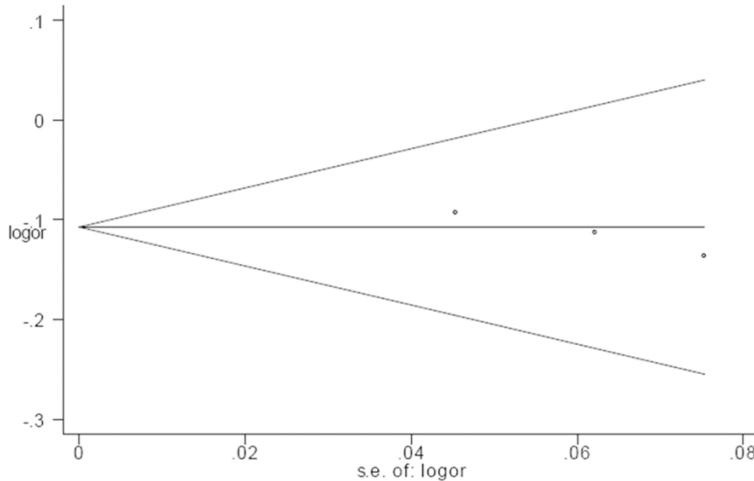


Figure 3. Begg's funnel plot of publication bias in selection of studies on the association between VEGF -634C/G polymorphism and ONFH risk in the additive model (C vs. G).

Table 3. The stability of the study was detected by sensitivity analysis through re-meta-analysis with one involved study excluded each time

Excluded study arm	OR	95% CI	P	I ² (%)
C vs. G				
Kim 2008	1.152	1.034-1.284	0.010	0
Lee 2012	1.135	1.038-1.241	0.005	0
Liu 2012	1.141	1.039-1.252	0.006	0
CC vs. GG				
Kim 2008	1.388	1.089-1.768	0.008	0
Lee 2012	1.322	1.077-1.623	0.008	0
Liu 2012	1.338	1.076-1.663	0.009	0
CG vs. GG				
Kim 2008	1.064	0.946-1.196	0.303	0
Lee 2012	1.103	1.002-1.213	0.046	0
Liu 2012	1.140	1.036-1.256	0.008	0
CG + CC vs. GG				
Kim 2008	1.084	0.992-1.184	0.076	0
Lee 2012	1.100	1.022-1.185	0.011	0
Liu 2012	1.122	1.040-1.210	0.003	0
CC vs. GG + CG				
Kim 2008	1.431	1.078-1.899	0.013	0
Lee 2012	1.273	1.007-1.611	0.044	0
Liu 2012	1.217	0.946-1.567	0.127	0

Statistical analysis

The strength of the association between VEGF -634C/G polymorphism and ONFH risk was assessed by an odds ratio (OR) with the corre-

sponding 95% CI. The statistical significance of OR was analyzed by a Z test, and *p* less than 0.05 was considered as statistically significant. We estimated with the dominant model (CC + CG vs. GG) and recessive model (CC vs. CG + GG) and then evaluated a codominant model (CC vs. GG and CG vs. GG). We also estimated the risks of the additive model (C vs. G). Heterogeneity between studies was tested through the Chi-square and I-square tests. If the *I*² value was greater than 50% and the *P* value was less than 0.05, the meta-analysis was considered as homogeneous. When there was no statistical heterogeneity, we used a fixed effects model. The OR were calculated using either fixed-effects models or, in the presence of heterogeneity, random-effects models.

The possible publication bias was examined visually in a funnel plot of log [OR] against its standard error (SE), and the degree of asymmetry was tested by Begg's test (*P*<0.05 was considered a significant publication bias). The stability of the study was also detected by sensitivity analysis, through re-meta-analysis with one involved study excluded each time. All statistical analyses were performed with Stata version 11.0 (Stata Corp, College Station, TX, USA).

Results

Literature search

The literature search yielded a total of 10 primary studies. These studies were included for full-text assessment, of which 7 were excluded for one of the following reasons: (1) irrelevant to our topic (n=2), (2) articles not providing avail-

able genotype frequency in both cases and controls (n=5). Overall, 3 eligible studies with 692 cases and 875 controls were considered in the meta-analysis [16-18]. A flow diagram of the study selection process is presented in **Figure 1**.

Study characteristics and quality assessment

The detailed characteristics of the included studies and the results of the quality assessment were summarized in **Table 1**. The earliest study was published in 2008, and the latest in 2012. By geographic location, two studies were conducted in Korea and one was in China. The number of subjects with ONFH in each study ranged from 160 to 312. Two included studies used polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) to detect genotypes, while the other one study used Taqman assay. The overall study quality averaged 7 stars on a scale of 0 to 9.

Association between VEGF -634C/G and ONFH

The distribution of the C allele frequency was 0.460 for the ONFH group and 0.407 for the control group (**Table 1**). The results of the meta-analysis and heterogeneity test are listed in **Table 2**. We analyzed the heterogeneity of five genetic models for all three studies, and found no significant amount of heterogeneity. Thus, the fix-effect model was used for synthesis of the data in the five genetic models.

When all the studies were pooled into meta-analysis, a significant association appeared between VEGF -634C/G polymorphism and ONFH risk (**Figure 2**) (for C vs. G: OR=1.141, 95% CI 1.055-1.235, p=0.001; for CC vs. GG: OR=1.345, 95% CI 1.124-1.610, p=0.001; for CG vs. GG: OR=1.106, 95% CI 1.018-1.202, p=0.017; for CG+CC vs. GG: OR=1.104, 95% CI 1.035-1.177, p=0.003; for CC vs. GG+CG: OR=1.294, 95% CI 1.051-1.593, p=0.015) (**Table 2**).

Publication bias and sensitivity analysis

Publication bias was determined by Begg's test and visualization of funnel plot. There was no evidence of publication bias (P=0.296) (**Figure 3**). Sensitivity analysis showed that excluding any one involved study from the pooled analy-

sis did not vary the results substantially, except for the study performed by Kim et al. in 2008, which can change the results of dominant model (CC + CG vs. GG) and codominant model (CG vs. GG) (**Table 3**).

Discussion

Although many pathophysiological models of bone necrosis have been established, the accurate mechanism of ONFH still has not been completely clarified. The vascular hypothesis appears to be the most persuasive among several confounding pathogenic mechanisms for ONFH, hypothesizing that a decrease in the local blood flow in the femoral head, owing to vascular obstruction by any means, plays an important role in the pathogenesis of ONFH [20, 21]. There are some studies in human and animal models of ONFH, indicating that vascular abnormalities have resulted in thrombosis associated with abnormal thrombophilic coagulopathy and hypofibrinolysis and embolism, which contribute to the development of ONFH [22-24]. As a major inducer of angiogenesis, VEGF is very important for bone formation processes, including blood vessel invasion and cartilage remodeling [10] and it has also been involved in bone repair [11, 21]. There are reports indicating that normal angiogenesis is pivotal for tissue repair, and that VEGF may be the major signal in the coupling of angiogenesis and osteogenesis during bone repair [9, 25]. The VEGF gene was reported to be polymorphic, especially in the promoter region, the 5'-UTR and in the 3'-UTR. It is reported that several transcription factor binding sites are found in the VEGF 5'-UTR and the transcriptional regulation of the gene is extremely complex [25]. Some studies have shown that polymorphisms within the 5'-UTR have lead to differences in VEGF expression between individuals, which can influence the etiology of a variety of pathological conditions, such as diabetic retinopathy [26] and type 1 diabetes mellitus [27].

In this study, we conducted a meta-analysis to assess the association of the VEGF -634C/G gene polymorphism with ONFH. The results of the meta-analysis indicated a significant association between the VEGF -634C/G gene polymorphism with the risk of ONFH. Although many etiological factors contributed to pathogenesis of ONFH and the pathologic changes of bone

marrow in ONFH were almost similar, it is generally accepted that the interruption of the circulation of blood is the final common pathway for the development of ONFH [28, 29]. Watson et al. [30] found that the VEGF -634G>C polymorphism was significantly associated with the VEGF expression of lipopolysaccharide(LPS)-stimulated peripheral blood mononuclear cells, and the VEGF production was the highest for the GG homozygotes and the lowest for the CC homozygotes. Therefore, we speculated that the decreased VEGF expression in the osteonecrotic bone area could affect angiogenesis, repair processes, the progression, and outcome of ONFH.

To the best of our knowledge, this is the first meta-analysis to estimate the association between VEGF -634C/G gene polymorphism with ONFH. We made sure to minimize the bias by means of study procedure. Not only did we search Pubmed, OVID and Web of Science to identify potential studies, but also we manually examined all reference lists from relevant studies. Publication bias was also absent, as determined by visualization of funnel plot and Begg's test. However, the possible limitations of our study must be considered. First, only three eligible studies involving 692 cases and 875 controls were included in the meta-analysis. Second, all of the studies were performed in Asian populations (Korean and Chinese). Further studies are needed in other ethnic populations because of possible ethnic differences of the VEGF -634C/G polymorphisms.

Conclusions

This meta-analysis supports a significant association between VEGF -634C/G polymorphism and ONFH risk. This finding needs further confirmation by trans-regional multicenter study with large sample in different ethnic populations, such as Caucasian and Austroloid.

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